Khuanjarat Choengpanya 2008: Glycone Specificity Engineering of Thai Rosewood β-Glucosidase for Mannose. Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Prachumporn Kongsaeree, Ph.D. 121 pages.

β-Glucosidases (EC 3.2.1.21) catalyze the hydrolysis of β-O-glucosidic linkages formed between D-glucose and aglycone group. The hydrolysis of glucoside substrates require the hydrogen bond interactions between glucose moiety and the active pocket amino acid residues for stabilization of transition state. Previous study has shown that the interaction of the hydroxyl group at C-2 position with amino acid residues in the glycone binding pocket was most important for transition state stabilization. Since glucose and mannose substrates are differ in the orientation of C-2 hydroxyl groups, which is equatorial in glucose and axial in mannose, it was interesting to study which amino acid residues are responsible for glycone specificity. Thai rosewood β-glucosidase or dalcochinase can hydrolyze *p*-nitrophenyl-β-D-glucopyranoside (*p*NP-Glc) with high efficiency, but show no activity toward *p*-nitrophenyl-β-D-mannopyranoside (*p*NP-Man), whereas most β-mannosidases show good hydrolytic activity toward mannoside substrate but cannot hydrolyze glucoside substrates. In this study, the amino acid residues that might be involved in glycone specificity of dalcochinase were studied. Molecular modeling and docking predicted that amino acid residues R90, W137, N181 and M369 of dalcochinase, corresponding to Q77, Q150, D206 and E374 of *Pyrococcus horikoshii* β-mannosidase, might be involved in glycone specificity. Therefore, four mutant forms of dalcochinase were generated, yielding R90Q, W137Q, N181D and M369E. The binding mode of sugar substrates, kinetic and transglycosylation properties of mutant enzymes were studied. The differences in K_m values of all mutant enzymes toward *p*NP-Glc were less than an order of magnitude (17.0, 24.0, 2.7, 11.9 mM for R90Q, W137Q, N181D and M369E, respectively) compared to wild-type recombinant dalcochinase (9.88 mM), suggesting that affinities of dalcochinase mutants for *p*NP-Glc substrate were not significantly affected by these mutations. The turnover rates of R90Q, W137Q and N181D indicated that residues R90, W137 and N181 of dalcochinase were important for catalysis activity. In agreement with the docking results, mutant M369E could hydrolyze *p*NP-Man, due to grater hydrogen-bond interactions between the residues and the mannose substrate, when compared to other mutants. In the transglucosylation studies, mutants N181D and M369E showed increased reactivity to ethanol, *n*-propanol and *n*-butanol acceptors, due to the low hydrolytic activities. In transmannosylation, wild-type dalcochinase could hydrolyze and transfer mannose to *n*-propanol and *n*-butanol. No alkyl-mannosides were obtained from all mutant enzymes. These results suggested that rearrangement of amino acid residues in the binding pocket of these mutant enzymes were not suitable for accommodation and hydrolysis of the mannoside substrates.

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20 / 5 / 2008